



(11) **EP 1 790 661 A3**

(12) **EUROPEAN PATENT APPLICATION**

(88) Date of publication A3:
22.08.2007 Bulletin 2007/34

(43) Date of publication A2:
30.05.2007 Bulletin 2007/22

(21) Application number: **06256018.0**

(22) Date of filing: **24.11.2006**

(51) Int Cl.:
C07K 14/395 (2006.01) **C12N 15/81** (2006.01)
C12N 5/10 (2006.01) **C12N 1/15** (2006.01)
C12N 1/21 (2006.01)

(84) Designated Contracting States:
**AT BE BG CH CY CZ DE DK EE ES FI FR GB GR
HU IE IS IT LI LT LU LV MC NL PL PT RO SE SI
SK TR**
Designated Extension States:
AL BA HR MK YU

(30) Priority: **24.11.2005 JP 2005339383**
01.11.2006 JP 2006297923

(71) Applicant: **National Institute of Advanced
Industrial Science
and Technology
Tokyo 100-8921 (JP)**

(72) Inventors:
• **Sahara, Takehiko,
c/o Hokkaido Center,
National Institute of
Sapporo-shi,
Hokkaido 062-8517 (JP)**

- **Gouda, Takako,
c/o Hokkaido Center,
National Institute of
Sapporo-shi,
Hokkaido 062-8517 (JP)**
- **Tochigi, Yuki,
c/o Hokkaido Center,
National Institute of
Sapporo-shi,
Hokkaido 062-8517 (JP)**
- **Ohgiya, Satoru,
c/o Hokkaido Center,
National Institute of
Sapporo-shi,
Hokkaido 062-8517 (JP)**

(74) Representative: **Maschio, Antonio
D Young & Co
120 Holborn
London EC1N 2DY (GB)**

(54) **A highly efficient secretory signal peptide and a protein expression system using the peptide thereof**

(57) This invention is directed to the identification of secretory signal peptides exhibiting higher secretion efficiency than conventional secretory signal peptides. Secretory signal peptides exhibiting higher secretion ability than the secretory signal peptides used in conventional

membrane and secretory protein expression systems are identified and isolated from membrane proteins and secretory proteins existing in the *Saccharomyces cerevisiae* genome.

EP 1 790 661 A3

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	<p>NAKAJIMA HARUSHI ET AL: "Expression of an 87-kD-beta-1,3-glucanase of Bacillus circulans IAM1165 in Saccharomyces cerevisiae by low-temperature incubation" BIOSCIENCE BIOTECHNOLOGY AND BIOCHEMISTRY, vol. 57, no. 12, 1993, pages 2039-2042, XP008077919 ISSN: 0916-8451 * abstract *</p> <p style="text-align: center;">-----</p>	1-16	<p>INV. C07K14/395 C12N15/81</p> <p>ADD. C12N5/10 C12N1/15 C12N1/21</p>
X	<p>KASAHARA SHIN ET AL: "Cloning of the Saccharomyces cerevisiae gene whose overexpression overcomes the effects of HM1-killer toxin, which inhibits beta-glucan synthesis" JOURNAL OF BACTERIOLOGY, vol. 176, no. 5, 1994, pages 1488-1499, XP002429932 ISSN: 0021-9193 * figure 4 *</p> <p style="text-align: center;">-----</p>	1-7,9-15	
X	<p>KOWALSKI LESLIE R Z ET AL: "Cold-shock induction of a family of TIP1-related proteins associated with the membrane in Saccharomyces cerevisiae" MOLECULAR MICROBIOLOGY, vol. 15, no. 2, 1995, pages 341-353, XP008077924 ISSN: 0950-382X * abstract *</p> <p style="text-align: center;">-----</p> <p style="text-align: right;">-/--</p>	1-7,9-15	<p>TECHNICAL FIELDS SEARCHED (IPC)</p> <p>C07K C12N</p>
<p>The present search report has been drawn up for all claims</p>			
Place of search		Date of completion of the search	Examiner
The Hague		5 July 2007	Kools, Patrick
CATEGORY OF CITED DOCUMENTS		<p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document</p>	
<p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p>			



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 06 25 6018

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
A	KJAERULFF ET AL: "Comparison of different signal peptides for secretion of heterologous proteins in fission yeast" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, ACADEMIC PRESS INC. ORLANDO, FL, US, vol. 336, no. 3, 28 October 2005 (2005-10-28), pages 974-982, XP005078711 ISSN: 0006-291X * abstract *	1-16	TECHNICAL FIELDS SEARCHED (IPC)
A	----- HOFMANN K J ET AL: "MUTATIONS OF THE ALPHA GALACTOSIDASE SIGNAL PEPTIDE WHICH GREATLY ENHANCE SECRETION OF HETEROLOGOUS PROTEINS BY YEAST" GENE (AMSTERDAM), vol. 101, no. 1, 1991, pages 105-112, XP002429933 ISSN: 0378-1119 * abstract *	1-16	
A	----- QING GUOLIANG ET AL: "Cold-shock induced high-yield protein production in Escherichia coli" NATURE BIOTECHNOLOGY, NATURE PUBLISHING GROUP, NEW YORK, NY, US, vol. 22, no. 7, July 2004 (2004-07), pages 877-882, XP002332227 ISSN: 1087-0156 * abstract * ----- -/--	1-16	
-The present search report has been drawn up for all claims			
Place of search The Hague		Date of completion of the search 5 July 2007	Examiner Kools, Patrick
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- & : member of the same patent family, corresponding document	

4

EPO FORM 1503 03.82 (P04C01)



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 06 25 6018

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
A	LI JINCAI ET AL: "Impediments to secretion of green fluorescent protein and its fusion from <i>Saccharomyces cerevisiae</i> " BIOTECHNOLOGY PROGRESS, vol. 18, no. 4, July 2002 (2002-07), pages 831-838, XP002429934 ISSN: 8756-7938 * abstract *	1-16	
A	EP 0 662 515 A1 (KOREA INST SCIENCE TECHNOLOGY [KR]) 12 July 1995 (1995-07-12) * the whole document *	1-16	
A	FRIGERIO G ET AL: "A <i>Saccharomyces cerevisiae</i> cyclophilin resident in the endoplasmic reticulum" JOURNAL OF MOLECULAR BIOLOGY, vol. 233, no. 1, 1993, pages 183-188, XP002441095 ISSN: 0022-2836 * the whole document *	9-16	
			TECHNICAL FIELDS SEARCHED (IPC)
The present search report has been drawn up for all claims			
Place of search The Hague		Date of completion of the search 5 July 2007	Examiner Kools, Patrick
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document</p>			

4
EPO FORM 1503 03.82 (P04C01)



European Patent
Office

Application Number

EP 06 25 6018

CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

- ☐ Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claim(s):
- ☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

- ☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.
- ☐ As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.
- ☒ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:
1-16 (all partially)
- ☐ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:



The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

Invention 1: Claims, 1-16 (all partially)

DNA molecules encoding a secretory signal peptide consisting of the amino acid sequence with ID NO 2, or with some variation and having secretory signal activity at 30 or 15 degrees Celcius. The peptide encoded by said DNA. Expression vector comprising said DNA and a foreign gene. A transformant transformed with said expression vector. Method of producing a protein using said transformant in a culture between 20 and 42 degrees Celcius, or 0 and 20 degrees Celcius.

Invention 2-9, Claims 1-16 (all partially)

As for subject 1, now for Seq ID No 4, 6, 8, 10, 12, 14, 16 and 18.

Invention 10-42, Claims 9-16 (all partially)

As for subject 1, now for the temperature being 15 degrees Celcius and the range being between 0 and 20 degrees Celcius. Only including sequences with Seq ID No 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, and 102.

**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

EP 06 25 6018

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

05-07-2007

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0662515	A1	12-07-1995	AT 197608 T 15-12-2000
		DE 69426289 D1 21-12-2000	
		DE 69426289 T2 12-04-2001	
		JP 2799348 B2 17-09-1998	
		JP 8047393 A 20-02-1996	
		US 5712113 A 27-01-1998	
